Detection of E. coli O157 and Salmonella species in some raw chicken meat cuts in Ismailia province, Egypt

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ABSTRACT

One hundred random fresh raw chicken breast and thigh samples (50 of each) were collected from an automatic poultry dressing plant in Ismailia city, Egypt for bacteriological and genetic detection of E. coli O157:H7 and Salmonella species. Out of the examined 100 samples, only 4 (4%) samples were contaminated with E. coli O157:H7, while non-O157 E. coli isolates represented 11% of E. coli isolates which were serotyped as O142:H5, O125:H5, O126 and O26 with incidence of 2, 4, 3, and 2% of the examined samples, respectively. On the other side, Salmonellae were detected in 11 samples (11%) and serologically identified as S. Typhi, S. Typhimurium and S. Enteritidis with the incidence of 1, 3, and 7%, respectively. Further, PCR investigation indicated that fliC gene was detected in all 4 isolates of E. coli O157 with incidence of 100%; while, fimA gene in 5 Salmonella isolates was detected in 4 (80%) of such examined isolates. Accordingly, it is obvious that raw chicken meat cuts were loaded with pathogenic foodborne bacteria exposing consumers to the high risk of food poisoning. Moreover, the results cleared that bacteriological traditional methods for detection of bacteria contaminating foods are labor-intensive and time-consuming, but PCR are more rapid and highly sensitive for identification of foodborne pathogens.

1. INTRODUCTION

In recent years, the poultry trade has skillfully maintained an incomparable growth rate, which may be attributed to the poultry production has comparatively low prices, highly nutritious, rapid development rates, and an excellent sort of further-processed products (Barbut, 2015). Chicken meat may be a common source of microbial foodborne pathogens like Salmonellae spp. and E. coli (Yulistiani et al., 2019) which can gain access to chicken meat through the whole cycle of production starting with scalding, defeathering and evisceration besides cross contamination which may come from adjacent birds and contaminated equipment. Throughout chicken slaughtering and preparation steps, fecal matter may contaminate carcasses due to evisceration faults (Mbata, 2005). Salmonellae and E. coli, as members of Enterobacteriaceae, are considered the 2nd most common serious causes of foodborne illness worldwide (FSIS, 2008). Escherichia coli are a large group of pathogenic and non-pathogenic enteric bacteria. Some pathogenic strains of E. coli can cause GIT disturbances through secreting heat-stable Shiga toxin, which commonly detected in the bowel of many animals reared for food production. The most commonly reported type of Shiga toxin producing E. coli (STEC) in USA is O157 strain. Some types of STEC frequently associated with severe disease, including symptoms of watery bloody diarrhea and some sort of kidney failure known as hemolytic uremic syndrome (HUS) (CDC, 2016). Salmonellae are widely distributed in nature; they were contributed among the causes of worldwide foodborne pathogens. According to an estimation made in 2010, Salmonellae were involved in more than 80 million cases of foodborne gastroenteritis every year worldwide, of which 155,000 were fatal (Majowicz et al., 2010). Polymerase Chain Reaction (PCR) is a laboratory investigative and confirmatory method through in vitro amplification of deoxyribonucleic acid (DNA); a sequences that are preferably unique to the organism of interest. Beginning with DNA of any origin (bacteria, viral, plant, animal) PCR can increase the amount of the targeted DNA sequences 10² to 10⁴ times. Genetic-based diagnostic methods characterized by specificity, sensitivity, and confirmatory assays compared to traditional methods (McKillip et al., 2004). Genes encoding H₇-specific flagellar antigen such as fliC gene of E. coli is a confirmatory indicator for E. coli O157:H7 (Carey et al. 2009). In addition, Salmonella enterica food poisoning serovars are characterized by fimbrial genes encoded by fim genes, where fimA gene is the major subunit (Zeiner et al., 2012). These genes make the virulence and support the bacterial pathogenicity leading to more serious hazards.

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Therefore, the present study was conducted to investigate the incidence of E. coli O\textsubscript{157:H}\textsubscript{7} and Salmonellae in the examined chicken meat samples by both traditional and molecular assays.

2. MATERIAL AND METHODS

2.1. Bacteriological examinations

2.1.1. Collection of samples:
One hundred samples of raw chicken breast and thigh (50 of each) “weighed about 250g/sample” were collected from an automatic poultry dressing plant in Ismailia city, Egypt. The collected samples were prepared as recommended by ISO 6887-1 (2017), then subjected to the following examinations:

2.1.2. Detection of E. coli was performed according to ISO 16649-2 (2001)
2.1.2.1. Serotyping of E. coli isolates was performed following Kok et al. (1996).
2.1.2.2. Detection of Salmonellae was performed according to ISO 6579-1 (2017).

2.2. Genetic detection of E. coli fliC gene, and Salmonella fimA gene

2.2.1. Genetic detection of E. coli fliC gene:
2.2.1.1. DNA extraction was performed according to Shah et al. (2009) using the QiAamp DNA Mini kit (Qiagen, Germany, GmbH) following manufacturer’s recommendations. Briefly, 200 µl of the prepared sample suspension was incubated with ten µl of proteinase K and 200 µl of lysis buffer at 56°C/10 min. After incubation, 200 µl of absolute ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer’s recommendations. Nucleic acid was eluted with 100 µl of elution buffer.
2.2.1.2. Oligonucleotide primer was purchased from Metabion (Germany) as listed in table (1).
2.2.1.3. PCR amplification was performed according to Fagan et al. (1999)
Primers were mixed in 25 µl reaction mixture consists of 12.5 µl PCR Master Mix (Takara, Japan), 1 µl of forward and reverse primers (20 pmol conc.), 4.5 µl of PCR grade water and 6 µl of DNA template. The reaction was performed in a calibrated adjusted 2720 thermalycler.

2.3. Molecular detection of Salmonella fimA gene:
2.3.1. DNA extraction was performed according to Shah et al. (2009)
An overnight bacterial culture (200 µl) was mixed with 800µl of distilled water and boiled for 10 min. The resulting solution was mixed with 0.63ml 10m MNTPs (Ahmed, 2009).
2.3.2. Oligonucleotide primer was purchased from Metabion (Germany) as listed in table (2).
2.3.3. DNA amplification was performed according to Singh et al. (2013).
The bacterial genomic DNA extracts were amplified by PCR in a mixture of 25 µl reaction mixture contained 13.25 sterile H\textsubscript{2}O, 0.25 ml 10x buffer, 0.63 ml 10m MNTPs, 1 ml 25Mm MgCl\textsubscript{2}, 1.25 µl primer F and primer R (20 pmol/ml conc.); and then, fill up to 25 µl PCR grade water.

2.3.4. Analysis of the PCR Products
The genomic copies of PCR products were separated by action of electrophoresis on 1.5% agarose gel (AppliChem, Germany, GmbH) soaked in 1x TBE buffer at 25°C passing 5V/cm electrical current. For gel analysis, twenty µl of the products was injected in each gel incision. 100 bp genetic ladders were utilized to verify the fragment size. The gel photo was captured by a gel documentation system and analyzed by computer software.

2.4. Statistical Analysis:
The recorded results were performed using the Analysis of Variance (ANOVA) test following Feldman et al. (2003).

3. RESULTS

3.1. Prevalence of E. coli
Results of the study verified detection of E. coli in six (6%) and nine (9%) breast and thigh samples, respectively. In addition, bacteriological and serotyping of the isolated E. coli strains revealed that 4 out of 15 isolated strains were E. coli O\textsubscript{157:H}\textsubscript{7} as recorded in table (3).

Gene band at 625bp as recorded in Table 1:

Figure 1: Positive gene amplification at 625bp for E. coli O\textsubscript{157:H}\textsubscript{7} gene. L. ladder. Neg. Control negative. Pos. Control positive. Lanes 1-4: positive isolates of O\textsubscript{157:H}\textsubscript{7} at 625 bp.
3.2. Prevalence of Salmonellae

The presence of Salmonella was investigated using standard bacteriological techniques, then examined genetically by conventional PCR technique to detect presence of fimA gene in the isolates. Results of the present study revealed detection of Salmonella spp. in eleven (11%) of the examined samples represented by seven (14%) and four (8%) breast and thigh samples, respectively as recorded in table (4).

<table>
<thead>
<tr>
<th>Table 4 Prevalence of Salmonellae isolated from the examined samples of chicken meat cuts</th>
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<tr>
<td>Examinèd chicken meat cuts samples (n=50)</td>
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<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Salmonella species</td>
</tr>
<tr>
<td>No.</td>
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<td>7</td>
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*Percent calculated according to the number of each examined samples (50).
**Percent calculated according to total number of the examined samples (100).

For confirmatory diagnosis of Salmonellae, fimA gene was investigated as recorded in Fig, (2), which showing that out of five examined isolates, four isolates carried fimA gene with prevalence of 80%.

![Fig. 2 Positive gene amplification at 512-hp for Salmonella fimA gene.](image)

4. DISCUSSION

The PCR assays are considered one of the most specific, rapid, and diagnostic tools for microbial food poisoning detection, especially for ensuring the safety and quality of food.

Results achieved in table (3) and Fig. (1) indicated that 100% of the identified E. coli O157:H7; serologically isolated from chicken meat samples were positive by application of PCR technique. Thus, there was complete agreement between the results of serological (traditional) methods and PCR technique for identification of E. coli O157:H7. These findings agree with those reported by Asensi and dos Reis et al. (2009), who reported that PCR matched with the results obtained by traditional classical methodology in less time and labor, made it a good alternative screening technique for screening and diagnosis of pathogenic foodborne contamination in a many samples such as imported and commercially produced poultry samples, getting benefit of rapid, specific and sensitivity.

In addition, the results of E. coli O157:H7 detection in raw chicken meat samples was previously reported by Saad et al. (2012), who found it with mean value of 1.4x10^2 CFU/g in their examined samples; and Guran et al. (2017), who detected E. coli O157:H7 in 5(1.3%) chicken meat samples out of 375 examined samples. They also recorded that fliC gene in their examined samples.

Salmonella is a crucial reason behind foodborne diseases related to increased morbidity and mortality through the world (Scallan et al., 2015). It was reported that foodborne Salmonella poisoning as the 2nd most recorded bacterial foodborne illness in the USA (Scallan et al., 2011), where it causes about 22.2% of foodborne diseases in China as reported by Wang et al. (2007).

Results achieved in table (4) indicates the prevalence of Salmonellae in the examined breast and thigh samples. The obtained results can be compared with those recorded by Jørgensen et al. (2002) (25%) of the examined chicken meat cuts, Molla and Mesfin (2003) (21.1%) of the examined chicken meat), Tibaijuka et al. (2003) (12.3% in raw chicken meat), Atia (2018) (Salmonella was isolated from 8% and 20% of breast and thigh samples, respectively), and Elisy (2019) (Salmonella was detected in 20 and 25% of the examined chicken breast and chicken thigh, respectively).

Results of molecular investigation of the presence of fimA gene in the examined Salmonella isolates were in agreement with Akeem et al. (2017), and Zhengquan et al. (2020), who detected fimA gene in all of Salmonellae strains (24 and 151 isolates, respectively). The specificity of the reaction is primarily dependent on careful selection of the oligonucleotide primers and primer annealing temperature.

5. CONCLUSION

The results concluded that, the PCR is one of the most promising techniques available for rapid detection of foodborne and environmental microorganisms. Raw chicken meat cuts are exposed to many contamination ways through its production cycle. Detection of such bacteria (E. coli O157:H7 and Salmonella) indicated that raw chicken meats can possess a health hazards to the consumers, furthermore, it encourages following more hygienic practices during production and processing.

6. REFERENCES

spp. Laboratory Services Division, Food Production and Inspection Branch, Agriculture and Agri-Food, Ottawa, Ontario, Canada.


